

In the claims:

17. (Amended 4X) A method for the *in vitro* proliferation of a multipotent neural stem cell comprising[the steps of]:

(a) [dissociating] obtaining cells derived from mammalian neural tissue containing at least one multipotent neural stem cell [to separate said multipotent neural stem cell from said tissue, said multipotent neural stem cell being] capable of producing progeny that are capable of differentiating into neurons and glia, including astrocytes, wherein said obtained cells have not been treated with serum in vitro;

(b) preparing a substantially serum-free culture medium containing at least one predetermined growth factor capable of inducing multipotent neural stem cell proliferation;

(c) preparing a [primary] cell culture by combining the cells obtained in (a) with the [adding said multipotent neural stem cell to said] culture medium prepared in (b) to induce proliferation of;

(d) proliferating] said multipotent neural stem cell [in said primary culture] to produce [progeny of said] multipotent neural stem cell progeny which includes daughter multipotent neural stem cells; and

([e]d) preparing a [secondary] subsequent cell culture by [transferring] combining said multipotent neural stem cell progeny [to] with fresh substantially serum-free culture medium containing at least one predetermined growth factor capable of inducing multipotent neural stem cell proliferation to proliferate said daughter multipotent neural stem cells to produce more progeny which include more daughter multipotent neural stem cells.

17. (Twice Amended) The method of Claim 17 wherein said culture medium of step (b) is [a] defined[culture medium and wherein said dissociated multipotent stem cell is not exposed to serum *in vitro*].

17. (Twice Amended) The method of Claim 17 wherein step ([e]d) is repeated.

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E 2
89. (Twice Amended) The method of Claim 17 wherein the multipotent neural stem cell progeny [passaged in] of steps (c) and (d) are in suspension.

90. (Twice Amended) The method of Claim 17 further comprising[the additional step of]:

([f]e) inducing [the] progeny [proliferated] produced in step ([e]d) to differentiate by plating said progeny on a fixed substrate.

E 3
91. (Twice Amended) The method of Claim [17] 89 further comprising[the additional step of]:

([f]e) inducing the progeny [proliferated] produced in step ([e]d) to differentiate in suspension by not [allowing said progeny to form clonally-derived neurospheres without] reinitiating proliferation.

E 4
92. (Twice Amended) The method of Claim 17 wherein the progeny produced in step ([d]c) grow in the form of a clonally-derived cluster of cells [neurosphere].

93. The method of Claim 93 wherein prior to step ([e]d) said neurosphere is dissociated to form a suspension of single cells which is transferred to said fresh culture medium in step ([e]d).

Please cancel claims 1-16, 19, 21-84 and 95 without prejudice or disclaimer.

Please add the following claims:

96. A method for the *in vitro* proliferation of a multipotent neural stem cell comprising:

(a) obtaining cells derived from juvenile or adult mammalian neural tissue containing at least one multipotent neural stem cell capable of producing progeny that are capable of differentiating into neurons and glia;

(b) preparing a culture medium containing at least one predetermined growth factor capable of inducing multipotent neural stem cell proliferation; and

(c) preparing a cell culture by combining the cells obtained in (a) to the culture medium prepared in (b) to induce proliferation of said multipotent neural

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stem cell to produce multipotent neural stem cell progeny which includes daughter multipotent neural stem cells.--

--15. The method of claim 96 further comprising:

(d) preparing a subsequent cell culture by combining said multipotent neural stem cell progeny with fresh culture medium containing at least one predetermined growth factor capable of inducing multipotent neural stem cell proliferation to proliferate said daughter multipotent neural stem cells to produce more progeny which include more daughter multipotent neural stem cells.--

--16. The method of claim 96 wherein said culture medium is substantially serum-free and wherein the cells obtained in (a) have not been treated with serum *in vitro*.--

--17. The method of claim 96 wherein said culture medium is defined.--

--18. The method of claim 96 wherein said growth factor is selected from the group consisting of epidermal growth factor, amphiregulin, fibroblast growth factor and transforming growth factor alpha.--

--19. A method for the *in vitro* proliferation of a human multipotent neural stem cell comprising:

(a) obtaining cells derived from human neural tissue containing at least one multipotent neural stem cell capable of producing progeny that are capable of differentiating into neurons and glia, wherein said obtained cells have not been treated with serum *in vitro*;

(b) preparing a substantially serum-free culture medium containing at least one predetermined growth factor capable of inducing multipotent neural stem cell proliferation; and

(c) preparing a cell culture by adding the cells obtained in (a) to the culture medium prepared in (b) to induce proliferation of said multipotent neural stem cell to produce multipotent neural stem cell progeny which includes daughter multipotent neural stem cells.--

--20. The method of claim 19 further comprising: